

WHAT IS CLAIMED IS:

1           1. A method of inhibiting expression of an endogenous cellular gene  
2 in a cell, the method comprising the step of:  
3                   contacting a first target site in the endogenous cellular gene with a first  
4 zinc finger protein, wherein the  $K_d$  of the zinc finger protein is less than about 25 nM;  
5                   thereby inhibiting expression of the endogenous cellular gene by at least  
6 about 20%.

1           2. The method of claim 1, wherein the step of contacting further  
2 comprises contacting a second target site in the endogenous cellular gene with a second  
3 zinc finger protein.

1           3. The method of claim 2, wherein the first and second target sites are  
2 adjacent.

1           4. The method of claim 3, wherein the first and second zinc finger  
2 proteins are covalently linked.

1           5. The method of claim 1, wherein the first zinc finger protein is a  
2 fusion protein comprising a regulatory domain.

1           6. The method of claim 5, wherein the first zinc finger protein is a  
2 fusion protein comprising at least two regulatory domains.

1           7. The method of claim 2, wherein the first and second zinc finger  
2 proteins are fusion proteins, each comprising a regulatory domain.

1           8. The method of claim 7, wherein the first and second zinc finger  
2 protein are fusion proteins, each comprising at least two regulatory domains.

1           9. A method of inhibiting expression of an endogenous cellular gene  
2 in a cell, the method comprising the step of:  
3                   contacting a target site in the endogenous cellular gene with a fusion zinc  
4 finger protein comprising six fingers and a regulatory domain, wherein the  $K_d$  of the zinc  
5 finger protein is less than about 25 nM;

6 thereby inhibiting expression of the endogenous cellular gene by at least  
7 about 20%.

1                           10.     The method of claim 1, wherein the cell is selected from the group  
2     consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

11. The method of claim 10, wherein the cell is a mammalian cell

12. The method of claim 11, wherein the cell is a human cell

1 15. The method of claim 1, wherein the endogenous cellular gene is  
2 VEGF.

1                   18. The method of claim 1, wherein the method further comprises the  
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,  
3 wherein the delivery vehicle comprises a liposome or a membrane translocation  
4 polypeptide.

1                   19. The method of claim 1, wherein the zinc finger protein is encoded  
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the  
3 method further comprises the step of first administering the nucleic acid to the cell in a  
4 lipid:nucleic acid complex or as naked nucleic acid.

1                   20. The method of claim 1, wherein the zinc finger protein is encoded  
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a  
3 promoter, and wherein the method further comprises the step of first administering the  
4 expression vector to the cell.

1                   21. The method of claim 20, wherein the expression vector is a viral  
2 expression vector.

1                   22. The method of claim 20, wherein the expression vector is a  
2 retroviral expression vector, an adenoviral expression vector, a DNA plasmid expression  
3 vector, or an AAV expression vector.

1                   23. The method of claim 20, wherein the zinc finger protein is encoded  
2 by a nucleic acid operably linked to an inducible promoter.

1                   24. The method of claim 20, wherein the zinc finger protein is encoded  
2 by a nucleic acid operably linked to a weak promoter.

1                   25. The method of claim 1, wherein the cell comprises less than about  
2  $1.5 \times 10^6$  copies of the zinc finger protein.

1                   26. The method of claim 1, wherein the target site is upstream of a  
2 transcription initiation site of the endogenous cellular gene.

1                   27. The method of claim 1, wherein the target site is adjacent to a  
2 transcription initiation site of the endogenous cellular gene.

1                   28. The method of claim 1, wherein the target site is adjacent to an  
2 RNA polymerase pause site downstream of a transcription initiation site of the  
3 endogenous cellular gene.

1                   29. The method of claim 1, wherein the zinc finger protein comprises  
2 an SP-1 backbone.

1                   30. The method of claim 29, wherein the zinc finger protein comprises  
2 a regulatory domain and is humanized.

1                   31. A method of activating expression of an endogenous cellular gene,  
2 the method comprising the step of:  
3                    contacting a first target site in the endogenous cellular gene with a first  
4 zinc finger protein, wherein the  $K_d$  of the zinc finger protein is less than about 25 nM;  
5                    thereby activating expression of the endogenous cellular gene to at least  
6 about 150%.

1                   32. The method of claim 31, wherein the step of contacting further  
2 comprises contacting a second target site in the endogenous cellular gene with a second  
3 zinc finger protein.

1                   33. The method of claim 32, wherein the first and second target sites  
2 are adjacent.

1                   34. The method of claim 33, wherein the first and second zinc finger  
2 proteins are covalently linked.

1                   35. The method of claim 31, wherein the first zinc finger protein is a  
2 fusion protein comprising a regulatory domain.

1                   36. The method of claim 35, wherein the first zinc finger protein is a  
2 fusion protein comprising at least two regulatory domains.

1                   37. The method of claim 32, wherein the first and second zinc finger  
2 proteins are fusion proteins, each comprising a regulatory domain.

1                   38. The method of claim 37, wherein the first and the second zinc  
2 finger protein are fusion proteins, each comprising at least two regulatory domains.

1                   39. A method of activating expression of an endogenous cellular gene,  
2 the method comprising the step of:  
3                    contacting a target site in the endogenous cellular gene with a fusion zinc  
4 finger protein comprising si: fingers and a regulatory domain, wherein the  $K_d$  of the zinc  
5 finger protein is less than about 25 nM;  
6                    thereby activating expression of the endogenous cellular gene to at least  
7 about 150%.

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1           40. The method of claim 31, wherein the cell is selected from the  
2 group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, or a  
3 fungal cell.

1           41. The method of claim 40, wherein the cell is a mammalian cell.

1           42. The method of claim 41, wherein the cell is a human cell

1           43. The method of claim 31, wherein expression of the endogenous  
2 cellular gene is activated to at least about 200-500%.

1           44. The method of claim 31, wherein the endogenous cellular gene is a  
2 selected from the group consisting of FAD2-1, EPO, GM-CSF, GDNF, VEGF, and LDL-  
3 R.

1           45. The method of claim 31, wherein the endogenous cellular gene is  
2 VEGF.

1           46. The method of claim 31, wherein the activation of gene expression  
2 prevents repression of gene expression.

1           47. The method of claim 35 or 37, wherein the regulatory domain is  
2 selected from the group consisting of a transcriptional activator, or a histone  
3 acetyltransferase.

1           48. The method of claim 31, wherein the method further comprises the  
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,  
3 wherein the delivery vehicle comprises a liposome or a membrane translocation  
4 polypeptide.

1           49. The method of claim 31, wherein the zinc finger protein is encoded  
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the  
3 method further comprises the step of first administering the nucleic acid to the cell in a  
4 lipid:nucleic acid complex or as naked nucleic acid.

1           50. The method of claim 31, wherein the zinc finger protein is encoded  
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a

3 promoter, and wherein the method further comprises the step of first administering the  
4 expression vector to the cell.

1                           51.    The method of claim 50, wherein the expression vector is a viral  
2 expression vector.

1                           52.     The method of claim 50, wherein the expression vector is a  
2 retroviral expression vector, an adenoviral vector, a DNA plasmid vector, or an AAV  
3 expression vector.

1                           53.    The method of claim 50, wherein the zinc finger protein is encoded  
2    by a nucleic acid operably linked to an inducible promoter.

1 55. The method of claim 31, wherein the cell comprises less than about  
2  $1.5 \times 10^6$  copies of the zinc finger protein.

1 57. The method of claim 31, wherein the target site is adjacent to a  
2 transcription initiation site of the endogenous cellular gene.

1 59. The method of claim 31, wherein the zinc finger protein comprises  
2 an SP-1 backbone.

1                           60.     The method of claim 59, wherein the zinc finger protein comprises  
2     a regulatory domain and is humanized.

3 contacting a first target site in the endogenous cellular gene with a first  
4 zinc finger protein;  
5 thereby modulating expression of the endogenous cellular gene.

1                           62.     The method of claim 61, wherein the step of contacting further  
2     comprises contacting a second target site in the endogenous cellular gene with a second  
3     zinc finger protein.

1                           64.     The method of claim 63, wherein the first and second zinc finger  
2     proteins are covalently linked.

1                   66.    The method of claim 65, wherein the first zinc finger protein is a  
2 fusion protein comprising at least two regulatory domains.

1                           69. A method of modulating expression of an endogenous cellular gene  
2 in a cell, the method comprising the step of:

3 contacting a target site in the endogenous cellular gene with a fusion zinc  
4 finger protein comprising six fingers and a regulatory domain;  
5 thereby modulating expression of the endogenous cellular gene.

1 71. The method of claim 70, wherein the cell is a mammalian cell

1                   72. The method of claim 71, wherein the cell is a human cell.

1                   73. The method of claim 61, wherein the endogenous cellular gene is a  
2 selected from the group consisting of VEGF, ER $\alpha$ , IGF-I, c-myc, c-myb, ICAM,  
3 Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R.

1                   74. The method of claim 61, wherein the endogenous cellular gene is  
2 VEGF.

1                   75. The method of claim 65 or 67, wherein the regulatory domain is  
2 selected from the group consisting of a transcriptional repressor, a transcriptional  
3 activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone  
4 deacetylase.

1                   76. The method of claim 61, wherein the method further comprises the  
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,  
3 wherein the delivery vehicle comprises a liposome or a membrane translocation  
4 polypeptide.

1                   77. The method of claim 61, wherein the zinc finger protein is encoded  
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the  
3 method further comprises the step of first administering the nucleic acid to the cell in a  
4 lipid:nucleic acid complex or as naked nucleic acid.

1                   78. The method of claim 61, wherein the zinc finger protein is encoded  
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a  
3 promoter, and wherein the method further comprises the step of first administering the  
4 expression vector to the cell.

1                   79. The method of claim 78, wherein the expression vector is a viral  
2 expression vector.

1                   80. The method of claim 78, wherein the expression vector is a  
2 retroviral expression vector, an adenoviral expression vector, a DNA plasmid expression  
3 vector, or an AAV expression vector.

1                   81.    The method of claim 78, wherein the zinc finger protein is encoded  
2    by a nucleic acid operably linked to an inducible promoter.

1                   82.    The method of claim 78, wherein the zinc finger protein is encoded  
2    by a nucleic acid operably linked to a weak promoter.

1                   83.    The method of claim 61, wherein the cell comprises less than about  
2     $1.5 \times 10^6$  copies of the zinc finger protein.

1                   84.    The method of claim 61, wherein the target site is upstream of a  
2    transcription initiation site of the endogenous cellular gene.

1                   85.    The method of claim 61, wherein the target site is adjacent to a  
2    transcription initiation site of the endogenous cellular gene.

1                   86.    The method of claim 61, wherein the target site is adjacent to an  
2    RNA polymerase pause site downstream of a transcription initiation site of the  
3    endogenous cellular gene.

1                   87.    The method of claim 61, wherein the zinc finger protein comprises  
2    an SP-1 backbone.

1                   88.    The method of claim 88, wherein the zinc finger protein comprises  
2    a regulatory domain and is humanized.

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